Mechanisms of adaptation in sheep to overcome silage intake depression induced by biogenic amines

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Effects of biogenic amines on silage intake and rumen fermentation during dietary changes were studied in sheep. Two direct-cut grass silages were prepared from a single grass sward, one untreated (WAS) and one treated with 4·0 litres formic acid (850 ml/l) per tonne (FAS). Diets of FAS, and FAS supplemented with 7·2 g biogenic amines/kg DM (FAS + A), were offered *ad libitum*, once daily to four rumen-cannulated, and four intact wethers in a repeated crossover design experiment. During a pre-period before each crossover, the animals were offered either the silage low in biogenic amines (FAS), or that containing moderate concentrations (WAS). During the first 4 d of the FAS + A treatment, the added biogenic amines tended to lower daily DM intake (DMI) and lowered significantly the DMI during the principal meal after feeding. This acute effect on DMI tended to be reduced when the sheep were previously preconditioned to amines by feeding WAS, and the acute DMI depression during the principal meal was significantly reduced. At the end of the 14 d FAS + A feeding period daily DMI was similar to that of the FAS treatment, but the daily pattern of intake remained different, with lower intake of FAS + A during the first 5 h after feeding, this being compensated for by the end of the day. Rumen fermentation tended to be less during the first 4 d that FAS + A was offered, due to the lower DMI and not due to the acute effect of amines. However, in the sheep unadapted to FAS + A, amine content in the rumen was higher than when the sheep were adapted for 14 d to FAS + A or WAS. Adaptation to FAS + A and feeding WAS during the pre-periods, increased the amine-degrading capacity of rumen fluid. In conclusion, in sheep unadapted to dietary amines, feeding amines will acutely lower DMI through reduced palatibility and most probably by stressing intermediary metabolism. Being preconditioned to amines slightly reduces the acute effect on daily DMI. Although the sheep adapted within 14 d to biogenic amines in the diet and increased daily DMI, there was clear evidence that amines have a negative effect on palatability.

Biogenic amines: Fixed intake: Grass silage: Sheep

Biogenic amines are thought to be one of the fermentation products which lower intake of ensiled forages, either by reducing palatability or by causing stress in intermediary metabolism after being absorbed (Beever & Reynolds, 1994). However, studies with sheep (Van Os *et al.* 1995*a*, 1996*a*) and dairy cows (Van Os *et al.* 1995*b*) showed that concentrations of amines (7 g/kg DM), comparable to those present in poor-quality grass silages, did not affect intake. In those studies, animals were adapted to the dietary treatment for 14 d before intake behaviour was monitored. However, some animals did show reduced silage intake during the first days after the change-over from a silage low in biogenic amine content to the same silage supplemented with biogenic amines. Possibly biogenic amines

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only affect silage intake in sheep that are not adapted or preconditioned to dietary amines. It was hypothesized that two mechanisms of adaptation to amines may be distinguished: (1) the animals may become accustomed to their taste, and (2) the increased degradation of amines by the rumen microbial mass (Van Os *et al.* 1995*c*) may prevent accumulation in the rumen, and subsequent absorption.

Therefore an experiment was carried out to investigate the acute effect of biogenic amines on silage intake in sheep not adapted to amines, to find out whether the animals adapt to the dietary amines, and if so, to identify the nature of the mechanisms of this adaptation.

MATERIALS AND METHODS

Dietary treatments and feeding

In June 1993, two grass silages were made from the second cut of a single sward of the Institute's permanent pastures of predominantly perennial ryegrass (*Lolium perenne*), fertilized with 450 kg N/1 $\times$ 10^4 m^2 (ha) per year. The grass was pre-wilted and harvested with a precision chop forage harvester. One part was ensiled untreated, and the other part was ensiled with 4·0 litres formic acid (850 ml/l) per tonne fresh material.

Three dietary treatments were used: the silage without additive (WAS), the formic acid preserved silage (FAS) and FAS supplemented with biogenic amines (FAS + A), at the rate of 1·2 g histamine, 2·8 g tyramine, 1·8 g putrescine and 1·4 g cadaverine/kg DM. The amounts of the individual amines added were fixed for FAS + A to achieve an amine content for this silage that was somewhat higher than that tested in a study by Van Os *et al.* (1996*a*). Histamine and tyramine were added in the hydrochloric form and putrescine and cadaverine as free bases. The amines were purchased from Sigma Chemical Company, St. Louis, MO, USA. All quantities of amines mentioned in the present paper refer to amines and not their hydrochlorides.

For preparation of FAS + A, the amines were dissolved individually in 7·5 litres distilled water at concentrations (g/l) of 16·8 for histamine, 39·2 for tyramine, 25·2 for putrescine and 19·6 for cadaverine. The total 30 litres of amine solutions was sprayed on 2 tonnes of FAS while mixing the silage in a feed mixing wagon. The WAS and FAS were similarly treated in the mixing wagon. After mixing, the amount of silage needed was stored in portions of 7 kg at $-20°$. The daily amount of the silages needed was placed at room temperature 24 h before feeding. The silages were offered to the sheep once daily (08.00 hours), after collection of the refusals of the previous day.

Animals and experimental design

Four non-fistulated 3-year-old (average live weights 80 kg), and four fistulated 2-year-old Texel wethers (average live weight 61 kg) were used. None of the wethers had consumed silage before. Each fistulated animal was equipped with a polyvinyl chloride rumen cannula (inner diameter 42 mm), 10 weeks before the start of pre-period A.

Pairs were made of one fistulated and one non-fistulated sheep, matching the fistulated sheep that was heaviest in body weight with the heaviest non-fistulated sheep. Likewise, three other pairs were made in descending order of body weight. Each pair was randomly assigned to the sequences of diet change-overs according to the experimental design given in Table 1. In this repeated crossover design, the periods 1 + 2 and 3 + 4 concerned the first and second crossover designs respectively. Measurements were made during the last 4 d of each pre-period (days 21–24), and during the first 4 d (days 1–4) and the last 4 d (days 11–

14) of each 14 d experimental period. The acute effects of biogenic amines on intake and rumen fermentation, as well as the extent of adaptation to dietary amines, were studied during the 14 d periods. The acute effect was defined as the difference between data measured on days 1–4 during which FAS + A was offered, and the data for FAS on days 11–14, within the same crossover design. The extent of adaptation to biogenic amines in FAS + A during the 14 d period was determined by comparing, within the crossover design, the values for FAS + A measured on days 11–14 with values for FAS measured on days 11–14. In order to determine the effect of previously having tasted amines (preconditioning), the sheep received, before each crossover, either the untreated silage (WAS) with a moderate biogenic amine content or FAS with a low amine content for a 24 d pre-period (period A or B). This effect of preconditioning was defined as the difference in the acute effect caused by the pre-period diet.

During the experiment, including the pre-periods, the sheep were kept in metabolism cages in an experimental unit where light was provided for 16 h daily. The animals had free access to water. Between the first and second crossover experiments, the sheep were taken from the metabolism cages and were allowed to pasture for 5 weeks during which they had free access to mineral blocks.

Measurements and sampling

From every 100 kg silage emptied from the feed mixing wagon a sample was taken manually. Refusals were sampled on each day of measurement. Both silage samples and pooled samples of refusals were stored at −20° until analysis.

The daily DM intake (DMI) was calculated as the difference between the amount offered and that refused. During four consecutive days, on days 1–4 and days 11–14 of each period, cumulative intake during the day was measured by reweighing the manger plus refusals at 0·5, 1, 1·5, 2, 3, 5, 7, 10, 16 and 24 h after feeding. On these days the time spent eating during the principal meal (first meal after feeding) was determined by visual observation and the amount eaten by reweighing the manger. The principal meal was considered to be finished when the sheep stopped eating and lay down, or withdrew from the manger and did not resume eating for at least 10 min.

On days 22 and 24 of the pre-period and on days 2, 4, 12 and 14 of each 14 d period, rumen fluid was taken from the ventral region by using a 100 ml syringe with rubber tube (length 0·5 m, inner diameter 7 mm). On these days 50 ml was taken just before feeding

Table 1. *Experimental design and assignment of pairs of sheep consisting of one fistulated and one non-fistulated animal, to the dietary treatments*

	Pair 1	Pair 2	Pair 3	Pair 4
Pre-period A (24 d)	WAS	WAS	FAS	FAS
Period 1 (14 d)	FAS	FAS + A	FAS	FAS + A
Period 2 (14 d)	FAS + A	FAS	FAS + A	FAS
	← On pasture (35 d) →			
Pre-period B (24 d)	FAS	FAS	WAS	WAS
Period 3 (14 d)	FAS	FAS + A	FAS	FAS + A
Period 4 (14 d)	FAS + A	FAS	FAS + A	FAS

WAS, grass silage preserved without additive; FAS, grass silage preserved with formic acid; FAS + A, FAS supplemented with 7·2 g biogenic amines/kg DM.

(08.00 hours), and 400 ml was taken 3 h after feeding (11.00 hours). Immediately after collection, the pH value of the fluid was measured and sub-samples (4·0 ml) were preserved with 4·0 ml 0·5 M-H_2SO_4 and 0·8 ml orthophosphoric acid (50 ml/l) for NH_3 and volatile fatty acid (VFA) analyses respectively.

The remaining fluid taken at 11.00 hours was used directly to determine *in vitro* the amine-degrading capacity of the rumen fluid, by adding biogenic amines and measuring the NH_3 liberated (Van Os *et al.* 1996*b*). Per sheep, four 100 ml glass tubes, equilibrated at 39°, were filled with 60 g unfiltered rumen fluid and closed with rubber stoppers permitting overpressure of fermentation gas to escape. Before closing the tubes, 0·8 ml of a freshly prepared solution of biogenic amine was added to two of the four tubes. This solution contained (g/l): 5·0 histamine, 17·0 tyramine, 8·0 putrescine and 8·0 cadaverine. Subsequently, the tubes were incubated in a shaking (100 rev./min) waterbath at 39°. Samples (2·0 ml) for NH_3 analysis were taken just before, and 1 h and 6 h after the start of the incubation. The net NH_3 production from the added amines was calculated as the difference between total NH_3 production and the endogenous NH_3 production of the rumen fluid.

Rumen content was sampled for biogenic amine analysis on days 1 and 13 of each 14 d period and on day 23 of the pre-periods. At 3 h after feeding (11.00 hours), about 300 g rumen content (liquid and solid phases) was withdrawn using a spring coil (Fadel *et al.* 1987). Exactly 50 g of the rumen content was immediately acidified with 30 ml TCA, (200 g/l) and stored at −20°. The remaining part was analysed for DM content.

Chemical analysis

Samples of the silages and refusals were dried at 70° and ground to pass a 1 mm screen. Both were analysed for DM and ash. The silages were additionally analysed for Kjeldahl-N, neutral-detergent fibre (NDF) and carbohydrates soluble in ethanol (400 ml/l) (SC), according to the methods used by Van Vuuren *et al.* (1989). Water extracts were made of silage samples (100 g silage/l) to determine lactic acid, VFA, alcohol, NH_3 concentrations and pH value. These components, as well as VFA and NH_3 concentrations in rumen fluid samples, were determined using the same methods as described by Van Os *et al.* (1996*b*).

The DM content of the silages was corrected for volatile components lost by oven-drying, as recommended by Dulphy *et al.* (1975). *In vitro* digestibility of the organic matter was determined by a modified Tilly and Terry method (Van der Meer, 1986). Biogenic amines were extracted from the silage as described by Van Os *et al* (1996*b*). The amines were extracted from the rumen contents by macerating the sample with the amount of TCA added for preservation. After centrifugation (20 min, 12 000 ***g***) of the macerates, biogenic amines were determined in the supernatant fraction by ion-exchange chromatography (Van Os *et al.* 1996*b*).

Statistical analysis

The data, normally distributed with homogeneous variance, were subjected to ANOVA using Genstat 5 (Genstat 5 Committee, 1987). Homogeneity of the variance was tested using Barlett's test (Steel & Torrie, 1980). Because the effect of fistulation on intake variables was not significant, this was excluded from the models used.

Three different effects of biogenic amines were statistically tested: the acute effect, the effect of being preconditioned and the effect of adaptation. The acute effect was tested by ANOVA on data from FAS + A measured on days 1–4 and data from FAS + A and FAS

measured on days 11–14 in the periods 1–4 (Table 1). Besides these factors (2 df) the effects of animal (7 df for all, and 3 df for fistulated sheep) and period (3 df) were included in the model. Differences between treatment means were compared by Student's *t* test. An acute effect of biogenic amines was declared when results from FAS + A measured on days 1–4 differed significantly from those for FAS measured on days 11–14.

The effect of preconditioning was considered to be present when feeding biogenic amines (WAS) during the pre-period significantly altered the extent of the acute effect in comparison with pre-feeding FAS. In the model used the effects of the pre-feeding treatment (1 df), animal (7 df or 3 df) and the effect of repetition of crossover (1 df) were included.

Adaptation to biogenic amines was derived from the change in measured variables during the 14 d period during which FAS + A was fed. Adaptation was statistically tested by comparing data of the treatments FAS and FAS + A both measured on days 11–14 in periods 1–4 (Table 1). Sheep were considered to be adapted to biogenic amines when results from FAS + A being given on days 11–14 did not significantly differ from those for FAS being given on days 11–14. The model included the effects of diet (1 df), animal (7 df or 3 df) and period (3 df).

Additionally, we tested whether intake characteristics and rumen fermentation of animals fed on diet WAS differed from those on FAS at the end of the pre-period. Therefore effect of diet (1 df) was tested in the model including effects of animals (7 df or 3 df) and pre-period (2 df).

RESULTS

Silage composition

Chemical compositions of WAS, FAS and FAS + A are presented in Table 2. The DM, crude protein (CP) and NDF contents, and organic matter digestibility did not significantly differ between WAS and FAS. Formic acid treatment limited the formation of lactic acid, VFA, NH_3 and biogenic amines, and increased residual SC in the silage. Addition of biogenic amines to FAS (FAS + A) resulted in an amine content slightly higher than that in the same type of silage used in the study of Van Os *et al.* (1996*a*). There were no other changes in silage composition compared with FAS.

Silage intake

Acute effect and preconditioning. Biogenic amines in FAS + A tended to reduce daily DMI during the first 4 d of feeding FAS + A (Table 3). This acute effect tended to be smaller when the sheep were preconditioned to biogenic amines by pre-feeding WAS. Similar, but significant effects were found for DMI during the principal meal. Though not significant, biogenic amines acutely lowered the rate of eating the principal meal, whereas being preconditioned to amines slightly lengthened the time spent eating.

The DMI during different time-intervals after feeding (Fig. 1) showed that biogenic amines acutely changed the daily intake pattern in sheep that were not preconditioned to amines (Fig. 1(a)). On days 1–4 of FAS + A feeding, the proportion eaten of the total daily DMI was lower ($P < 0{\cdot}05$) during the first 5 h after feeding than with FAS on days 11–14. However, this depressed intake was compensated by an increase ($P < 0{\cdot}05$) of the proportional DMI during the night (14–24 h after feeding). No acute change of the intake pattern was observed in the preconditioned sheep (Fig. 1(b)). Although proportional DMI

Table 2. *Chemical composition and fermentation characteristics (g/kg DM) of grass silages preserved without additive (WAS) or with formic acid (FAS) and FAS after amine addition (FAS + A)*

	WAS	FAS	FAS + A
Dry matter (g/kg fresh wt)	184	207	210
pH	3·8	3·9	3·9
Crude protein (N × 6·25)	159	162	166
NH_3-N (g/kg N_{total})	84	58	62
Neutral-detergent fibre	476	432	446
Soluble carbohydrates	33	104	95
Ash	137	133	131
OM digestibility (*in vitro*)	68·2	68·6	68·0
Lactic acid	110·1	65·4	72·1
VFA	54·8	21·5	22·9
Acetic acid	47·5	20·6	22·2
Propionic acid	2·9	0·4	0·4
Butyric acid	2·2	0·5	0·3
Alcohols	20·5	5·4	5·9
Amines	5·2	1·4	8·9
Cadaverine	1·4	0·3	1·9
Histamine	0·7	0·1	1·3
Putrescine	1·0	0·3	1·9
Tyramine	2·1	0·7	3·7

VFA, voltaile fatty acids; OM, organic matter expressed as % digested.

of FAS + A on days 1–4 was slightly lower during the first 5 h after feeding and slightly higher at the end of the day, the pattern of intake did not differ significantly from that of FAS on days 11–14.

Adaptation. No significant differences in daily DMI were observed between FAS + A and FAS on days 11–14 (Table 4). Although the sheep increased the DMI of the principal meal of FAS + A on days 1–4 over the 14 d experimental period, they did not fully adapt to the level of FAS on days 11–14. The DMI of the principal meal of FAS + A on days 11–14 remained lower ($P < 0·05$). The reduced DMI resulted from a combination of a slightly shorter time spent eating and a slightly lower intake rate. Although similar daily DMI of FAS + A and FAS on days 11–14 suggests full adaptation to amines, the proportional DMI over the day differed between the silages (Fig. 2). During the first 5 h after feeding DMI of FAS + A remained slightly depressed, but was compensated for by the end of the day.

Intake characteristics of WAS and FAS at the end of the pre-periods (Table 4) showed that daily DMI of WAS, with moderate biogenic amine content, was lower than that of FAS. This was mainly caused by the lower ($P < 0·05$) proportional DMI during the night (Fig. 2).

Rumen fermentation

Acute effect and preconditioning. Before feeding, no acute effects of biogenic amines were found on rumen fermentation variables during days 1–4 of FAS + A feeding. Values were comparable with those measured before feeding FAS + A on days 11–14 (Table 6). In samples taken 3 h after feeding, amines acutely lowered rumen NH_3 content and the proportion of propionic acid in the rumen VFA pool (Table 5). Being preconditioned to

Table 3. *The acute effect of biogenic amines (A) on dry-matter intake (DMI) and intake characteristics during the principal meal, in sheep fed on grass silage treated with formic acid (FAS), and the influence on this acute effect of being preconditioned to amines by pre-feeding with untreated grass silage (WAS)*†

Pre-fed diet ...	WAS			FAS			Statistical significance of:			
Dietary treatment ...	FAS + A	FAS + A	FAS	FAS + A	FAS + A	FAS	acute		precon-	
Measurement days ...	1–4	11–14	11–14	1–4	11–14	11–14	effect‡	SED§	ditioning‡	SED§
Daily DMI (g)	1331	1354	1368	1333	1352	1386	0·07	34	0·09	17
Principal meal:										
DMI	164	175	188	134	151	182	**	17	*	10
duration (min)	20	18	19	16	19	20	NS	2·2	NS	2·3
intake rate (g DM/min)	8·3	10·0	9·9	8·2	8·2	9·6	NS	1·0	NS	1·3

SED, standard error of difference.
NS, $P > 0{\cdot}1$. For tendencies, ($0{\cdot}05 < P < 0{\cdot}10$) probabilities are given.
*$P < 0{\cdot}05$, **$P < 0{\cdot}01$.
†For details of treatments and procedures, see Tables 1 and 2.
‡For definitions of the determined effects, see pp. 402–403.
§35 df for the acute effect; 6 df for the effect of being preconditioned.

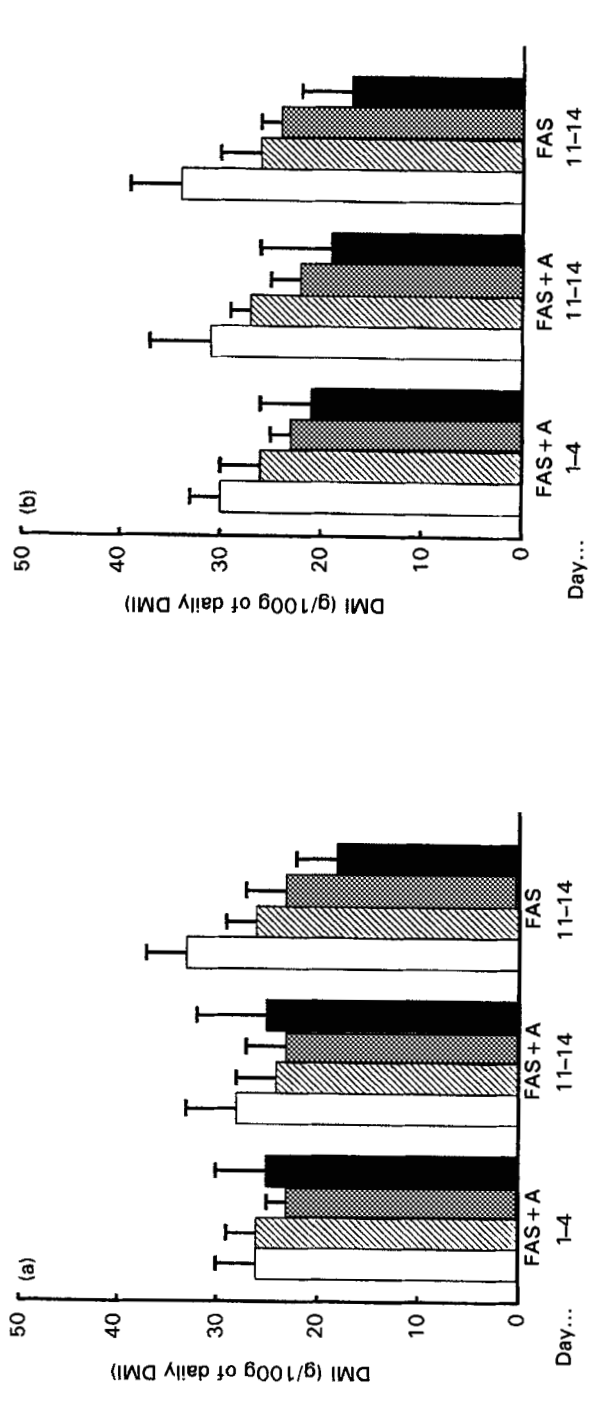

Fig. 1. Dry-matter intake (DMI) at different time intervals after feeding, in sheep offered a formic acid-preserved grass silage (FAS) or FAS supplemented with biogenic amines (FAS + A) for a period of 14 d. Measurements were made over 4 d at the beginning (days 1–4) or the end (days 11–14) of the feeding period and are means for eight sheep with their standard errors represented by vertical bars. During a pre-period sheep were fed on silage with (a) a low amine content (FAS) or (b) a moderate biogenic amine content (WAS). Time intervals after feeding were: (□), 0–5 h; (▨), 5–10 h; (▩), 10–14 h; (■), 14–24 h.

Table 4. *Daily dry matter intake (DMI) and intake activities during the principal meal in sheep on days 11–14 of the experimental period, when adapted to a grass silage preserved with formic acid (FAS) or FAS supplemented with biogenic amines (FAS + A), and on days 21–24 of the pre-period when adapted to FAS or untreated silage (WAS)*†

	FAS + A *v.* FAS				WAS *v.* FAS			
Dietary treatment ...	FAS + A	FAS	Statistical significance	SED‡	WAS	FAS	Statistical significance	SED‡
Daily DMI (g)	1353	1378	NS	31	1270	1451	*	53
Principal meal:								
DMI (g)	163	185	*	9	180	188	NS	18
duration (min)	18	19	NS	0·9	21	20	NS	2·5
intake rate (g DM/min)	9·1	9·8	NS	0·6	8·5	9·3	NS	1·6

SED, standard error of difference.
NS, $P > 0{\cdot}10$; $*P < 0{\cdot}05$.
†For details of treatments and procedures see Tables 1 and 2.
§6 df for WAS *v.* FAS; 20 df for FAS *v.* FAS + A.

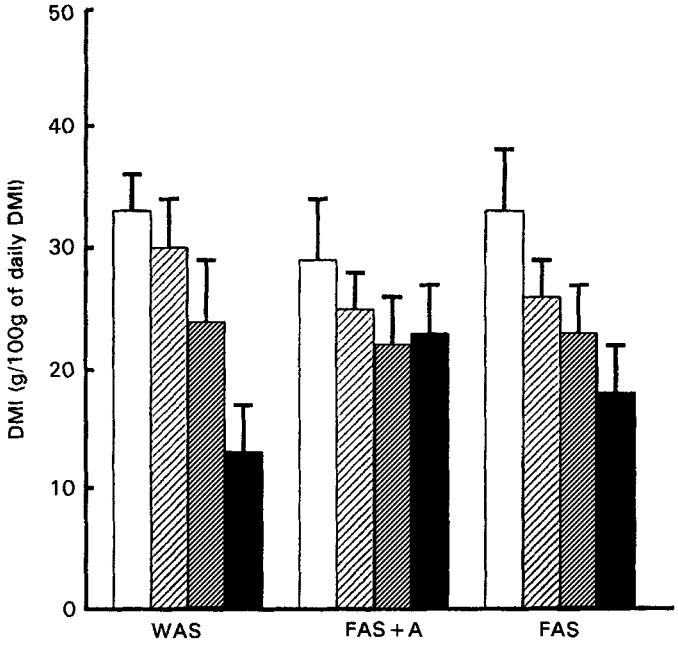

Fig. 2. Dry-matter intake (DMI) at different time intervals after feeding, in sheep offered a formic acid-preserved grass silage (FAS) or FAS supplemented with biogenic amines (FAS + A) on days 11–14 of a 14 d feeding period, and in the same sheep offered untreated silage (WAS) on days 21–24 of a pre-feeding period. Values are means for eight sheep with their standard errors represented by vertical bars. Time intervals after feeding were: (□), 0–5 h; (▨), 5–10 h; (■), 10–14 h; (■), 14-24 h.

amines by pre-feeding WAS did not significantly alter the acute effect on NH_3 content or the proportion of propionic acid in the rumen.

Adaptation. On days 11–14 of feeding FAS + A, no influences of biogenic amines on any of the measured rumen fermentation characteristics were found, either before or 3 h after feeding (Table 6).

Table 5. *The acute effect of biogenic amines (A) on rumen-fluid pH, ammonia and volatile fatty acid (VFA) content 3 h after feeding, in sheep fed on grass silage treated with formic acid (FAS)*†

Dietary treatment ...	FAS + A	FAS + A	FAS	Significance of the	
Measurement days ...	1–4	11–14	11–14	acute effect	SED
NH_3 (mg/l)	175	229	249	*	29
VFA (mmol/l)‡	89·0	92·0	97·6	NS	5·14
Acetic acid§	62·9	61·5	62·1	NS	0·70
Propionic acid§	20·9	23·7	23·3	*	1·19
Butyric acid§	11·0	10·2	10·4	NS	0·51
pH	6·56	6·50	6·54	NS	0·11

SED, standard error of difference, 14 df.
NS, $P > 0{\cdot}10$; *$P < 0{\cdot}05$.
†For details of treatments and procedures, see Tables 1 and 2.
‡Total VFA concentration includes acetic acid, propionic acid, methyl propionic acid, butyric acid, 2- and 3-methyl butyric acid and valeric acid.
§mol/100 mol.

Table 6. *Rumen-fluid pH, ammonia and volatile fatty acid (VFA) content before, and 3 h after feeding in sheep on days 11–14 of the experimental period, when adapted to a grass silage preserved with formic acid (FAS) or FAS supplemented with biogenic amines (FAS + A), and on days 21–24 of the pre-period when adapted to FAS or untreated silage (WAS)*†

	FAS + A *v.* FAS				WAS *v.* FAS			
Dietary treatment ...	FAS + A	FAS	Statistical significance	SED‡	WAS	FAS	Statistical significance	SED§
Before feeding:								
NH_3 (mg/l)	145	143	NS	10·5	123	120	NS	6·2
VFA (mmol/l)§	66·7	72·1	NS	3·3	61·5	77·8	**	9·6
Acetic acid‖	65·8	67·6	NS	1·4	68·4	67·2	NS	0·6
Propionic acid‖	19·8	19·2	NS	1·3	17·1	18·7	**	0·2
Butyric acid‖	10·5	9·9	NS	0·2	10·3	9·8	NS	0·2
pH	6·78	6·82	NS	0·06	6·92	6·69	*	0·05
3 h after feeding:								
NH_3 (mg/l)	229	249	NS	19·1	232	200	*	7·4
VFA (mmol/l)§	92·0	97·6	NS	2·8	88·0	97·6	**	0·7
Acetic acid‖	61·5	62·1	NS	0·3	61·6	63·0	NS	0·5
Propionic acid‖	23·7	23·2	NS	0·7	22·0	20·3	NS	1·1
Butyric acid‖	10·1	10·4	NS	0·3	11·8	10·5	*	0·2
pH	6·50	6·54	NS	0·05	6·69	6·63	NS	0·10

SED, standard error of difference.
NS, $P > 0{\cdot}10$; *$P < 0{\cdot}05$, **$P < 0{\cdot}01$.
For details of treatments and procedures, see Tables 1 and 2.
‡2 df for WAS *v.* FAS, 8 df for FAS *v.* FAS + A.
§Total VFA concentration including acetic acid, propionic acid, methyl propionic acid, butyric acid, 2- and 3-methyl butyric acid and valeric acid.
‖mol/100 mol.

In sheep pre-fed with WAS, rumen fermentation remained different from that of sheep that received only FAS in the pre-period. Notably the pH value before feeding was higher with WAS and total VFA content was lower, with a lower molar proportion of propionic acid. In samples taken after feeding, rumen NH_3 was higher with WAS and VFA content was lower, with a higher molar proportion of butyric acid in the VFA pool.

Table 7. *The acute effect of biogenic amines (A) on rumen amine content (mg/kg DM), 3 h after feeding, in sheep fed on grass silage treated with formic acid (FAS), and the influence on this acute effect of being preconditioned to amines by pre-feeding with untreated grass silage (WAS)*†

Pre-fed diet ...	WAS			FAS			Statistical significance of:			
Dietary treatment ...	FAS + A	FAS ● A	FAS	FAS + A	FAS + A	FAS	acute		precon-	
Measurement days ...	1	14	14	1	14	14	effect	SED‡	ditioning	SED‡
Cadaverine	254	16	22	229	18	16	**	56	NS	49
Histamine	295	17	14	340	11	12	**	63	NS	43
Putrescine	46	31	32	61	34	30	*	7	NS	16
Tyramine	468	15	34	382	18	21	**	67	NS	151

SED, standard error of difference.
NS, $P > 0{\cdot}10$; *$P < 0{\cdot}05$, **$P < 0{\cdot}01$.
†For details of treatments and procedures, see Tables 1 and 2.
§14 df for the acute effect, 2 df for the effect of being preconditioned.

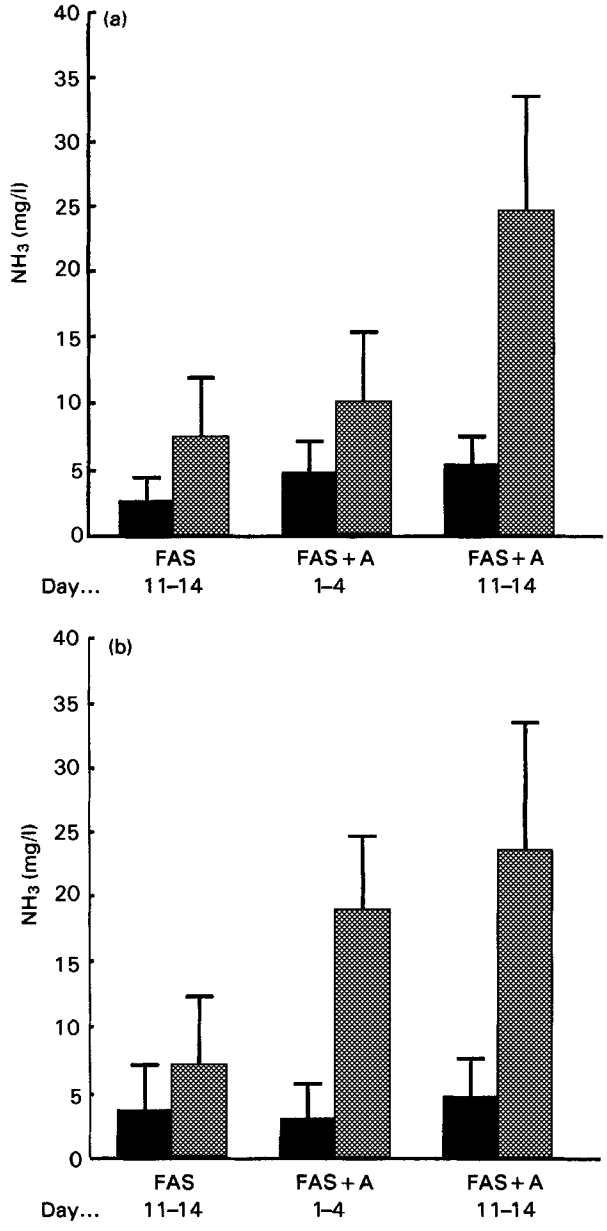

Fig. 3. Production of ammonia from biogenic amines added to rumen fluid, after (■) 1 h and (▩) 6 h of *in vitro* incubation. Rumen fluid was withdrawn from sheep offered a formic acid-preserved silage (FAS) on days 11–14 of a 14 d feeding period, and from sheep offered FAS supplemented with biogenic amines (FAS + A) on days 1–4 and 11–14 of a 14 d feeding period. In a pre-period sheep were fed on silage with (a) a low amine content (FAS) or (b) a moderate biogenic amine content (WAS). Values are means for four sheep with their standard errors represented by vertical bars.

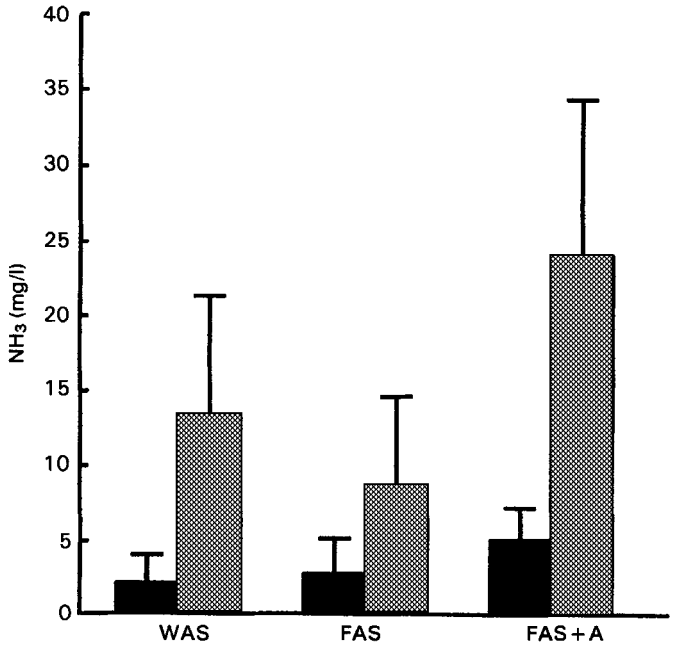

Fig. 4. Production of ammonia from biogenic amines added to rumen fluid, after (■) 1 h and (▩) 6 h of *in vitro* incubation. Rumen fluid was withdrawn from sheep offered a formic acid-preserved grass silage (FAS) or FAS supplemented with biogenic amines (FAS + A) on days 11–14 of a 14 d feeding period, and on days 21–24 of a pre-period when untreated silage (WAS) was offered. Values are means for four sheep with their standard errors represented by vertical bars.

Rumen biogenic amine content

Acute effect and preconditioning. During the first day of FAS + A feeding, a significant rise of all individual amines was observed in the rumen, 3 h after feeding (Table 7). The highest increase was found for tyramine followed by cadaverine and histamine. No significant influence of preconditioning to amines was associated with this acute rise of amines in the rumen.

The biogenic amine-degrading capacity of the rumen fluid, measured by NH_3 production from added amines, was low in sheep offered FAS on days 11–14 and offered FAS + A on days 1–4 (Fig. 3). The latter was more pronounced in sheep that were not preconditioned (Fig 3(a)). Preconditioning by pre-feeding WAS (Fig. 3(b)) tended to increase ($P = 0{\cdot}08$) amine degradation in the rumen fluid of sheep offered FAS + A on days 1–4.

Adaptation. When the sheep were offered FAS + A, the biogenic amine-degrading capacity of the rumen fluid taken on days 11–14, was higher ($P < 0.01$) than that of the fluid from sheep offered FAS on days 11–14 (Fig. 4). On day 14 only traces (< 35 mg/kg DM) of the four biogenic amines were found in the rumen content of sheep offered FAS + A on day 14.

The additional comparison of WAS and FAS at the end of the pre-periods showed a slightly higher amine-degrading capacity of the rumen fluid on WAS than on FAS (Fig. 4). Nevertheless, rumen biogenic amine content on WAS was similar to that on FAS and contained only traces of amines.

DISCUSSION

The acute effect of biogenic amines

Our previous studies on the influence of biogenic amines on grass silage intake in sheep and dairy cows showed that amines apparently had no impact on chemostatic intake control and only tended to impair silage palatability (Van Os *et al.* 1995*a*,*b*, 1996*a*). In those studies measurements were carried out with animals that had been adapted to the diets containing amines for 14 d. In the present study, a grass silage containing biogenic amines was fed to sheep that had never been fed on silage before and were assumed to be unadapted and not preconditioned to biogenic amines. In these sheep, it was found that a sudden increase of biogenic amines in the diet depressed DMI, tended to depress rumen fermentation rate and increased biogenic amine concentrations in the rumen. This acute effect on DMI is attributed to negative effects on palatability of this combination of amines deduced from the persistent tendency towards lower intake rates in adapted sheep (Van Os *et al.* 1995*a*, 1996*a*) together with feedback signals from chemostatic intake regulation as a result of the high amine concentrations in the rumen.

The low capacity of rumen microbiota to degrade amines in unadapted sheep resulted in the accumulation of biogenic amines in the rumen during the first 3 h after feeding FAS + A. This accumulation possibly continued for some days during which the rumen microbiota were not fully adapted to be able to degrade the ingested amines. Tveit *et al.* (1992) found increasing concentrations of amines in the rumen of dairy cows 3 d after a changeover from hay to a silage containing amines. Therefore, the increase in biogenic amines in the rumen may possibly act on the chemostatic intake control through local effects in the rumen, or after absorption, by their impact on the intermediary metabolism.

In the present study, it was assumed that rumen fermentation was inhibited as a result of a lower DMI in the sheep unadapted to FAS + A, rather than through an acute negative effect of amines on microbial activity. This is because adding biogenic amines to the rumen content of unadapted, hay-fed sheep resulted in increased NH_3 and VFA production and unaltered gas production during *in vitro* fermentation (Van Os *et al.* 1995*c*).

It is possible that signals of satiation are generated through chemoreceptors in the rumen, detecting the increased concentrations of amines. However, no chemoreceptors for biogenic amines have been reported to be present in the rumen. Nevertheless, negative effects of tyramine on the development of rumen epithelium have been found (Kutas *et al.* 1986), while Dain *et al.* (1955) observed a decrease in rumen motility by histamine and tyramine, but this decreased motility was found in combination with rumen acidosis. Histamine is not absorbed by the rumen epithelium (Kay & Sjaastad, 1974), but whether the other biogenic amines are absorbed from the rumen is unknown. The acute effects of biogenic amines on intake at the ruminal level are probably limited. It is more likely that the high concentrations of amines in the rumen resulted in an increased flow of amines to the lower stomachs and intestines. Possibly, amines lead to increased gastrin secretion in the sheep abomasum, as observed in the rat stomach (Lichtenberger *et al.* 1982). Gastrin has been reported to reduce rumen motility and depress intake in sheep (Grovum, 1981). Furthermore, histamine infused into the abomasum of sheep depressed feed intake and increased their respiration rate (Neumark, 1967). It must, however, be noticed that the quantity of histamine infused by Neumark (1967) was about double that expected to be found in the abomasum of our unadapted sheep fed on FAS + A.

If concentrations of amines in the intestines exceed the capacity of the amine oxidizing enzymes in the intestinal wall, they will be absorbed causing stress in intermediary metabolism (Joosten, 1988; Sattler *et al.* 1988). In goats, intravenously injected tyramine

stimulates the release of catecholamines and corticoids (Forbes *et al.* 1994). The sheep in the present study did not exhibit symptoms of clinical intoxication such as increased respiration on sudden intake of biogenic amines. A subclinical effect on chemostatic regulation of DMI through increased absorption of biogenic amines however cannot be excluded. This is supported by the depressed DMI on rumen infusion of the same combination of amines in sheep adapted to silages with a low content of biogenic amines (Buchanan-Smith & Phillip, 1986) and by the lower DMI in cows on the introduction to the rumen of a high dose of putrescine (Lingaas & Tveit, 1992).

Preconditioning to biogenic amines

Buchanan-Smith (1990), suggested that sheep might become accustomed to the taste of biogenic amines. In the present study, the sheep were preconditioned to amines by pre-feeding WAS. Preconditioning was deduced from a decrease in the acute effect of amines lowering daily DMI and the DMI during the principal meal and from the similarity of the daily intake pattern of FAS + A on days 1–4 with that of FAS on days 11–14, when pre-feeding WAS. That this effect of preconditioning was only due to getting used to the taste of amines is doubtful, because pre-feeding amines also slightly increased the amine-degrading capacity of the rumen microbiota. A faster degradation of amines should have lowered the amount of amines in the rumen during days 1–4 that FAS + A was fed, subsequently reducing the flow of amines to the lower intestines and possibly to intermediary metabolism. Although expected, pre-feeding WAS did not lower the acute high concentrations of amines in the rumen on the first day of feeding FAS + A. This, however, cannot be ruled out on days 2–4 of feeding FAS + A, because no measurements of rumen amine content were made.

Adaptation to biogenic amines

The present study confirmed that sheep adapt to biogenic amines in the diet. The acute effects of biogenic amines found on DMI, rumen fermentation and the rumen amine content disappeared within the 14 d period of feeding FAS + A. The key role in adaptation is probably the increase of the amine-degrading capacity of the rumen microbiota, preventing accumulation of amines in the rumen which may affect chemostatic intake control.

Although daily DMI was similar for sheep adapted to FAS + A and FAS, the DMI during the principal meal of FAS + A and during the first 5 h after feeding remained lower in sheep that were not preconditioned and slightly lower in preconditioned sheep. Since no influence of amines on metabolic intake control was expected in sheep adapted to FAS + A, this indicates the persistence of a slight negative effect of amines on palatability, confirming the results of Van Os *et al.* (1995*a*, 1996*a*). A similar intake pattern, with reduced intake just after feeding and compensation at the end of the day has also been reported by Deswysen *et al.* (1991) with grass silage supplemented with a methionine hydroxy analogue. These authors also attributed this shift in intake towards the later part of the day to the unpalatability of the added agent. In dairy cows having high intakes of grass silage, this slight negative effect of amines on palatability did not result in lower daily DMI or milk yield (Van Os *et al.* 1995*b*). Besides, it is expected that the motivation to eat in lactating dairy cows will be higher than in sheep fed at maintenance.

In conclusion, we demonstrated that in sheep which are not adapted to dietary biogenic amines, feeding a silage containing amines depresses DMI directly through reduced

palatability and most probably through their effects on chemostatic intake regulation. In sheep preconditioned to amines by pre-feeding a silage containing amines, this acute effect was less. When a diet containing biogenic amines was fed for a longer period, the sheep adapted relatively quickly by increasing the rate of degradation of amines in the rumen and partly by becoming accustomed to their taste. Therefore the generally persistent lower DMI of untreated silages (type WAS) compared with hay or formic acid preserved silage (type FAS) cannot be attributed to the effect of biogenic amines *per se* on intake control.

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